REMARKS/ARGUMENTS

This is a preliminary amendment in a RCE application. The Office Action mailed March 29, 2007 has been carefully reviewed. Reconsideration of this application, as amended and in view of the following remarks, is respectfully requested. Claims 1-17 appeared in the application as filed. Claims 1-10 are withdrawn from consideration as a response to a restriction requirement. Claims 12 and 14 have been cancelled. The claims presented for examination are: amended claims 11, 13, and 15-17.

35 U.S.C. § 112 Rejection

In numbered paragraph 3 of the Office action mailed March 29, 2007, claims 11-17 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention because claim 11 recites, "arraying ... fragments ... into groups." The Office action mailed March 29, 2007 stated, "It is not clear whether the step intends to purify/separate actual DNA fragments into groups (e.g., into different tubes, parts of an array, etc.) or 'virtually' array fragments by using a computer program. As the relationship between the method steps is not clear, clams 11-17 are indefinite."

Applicants have amended the claims to state, "virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size where n is an odd number" and "providing fragments of length n (n-mers)

of defined size where n is an odd number that correspond to said virtual fragments."

Applicants believe that since the amendment described above clarifies that there is a step of "virtually preselecting .... to virtually break said user-defined sequence into virtual fragments" and a step of "providing fragments...." It is clear that the first step is a virtual step and the second step is an actual step.

Applicants believe that the amendments overcome the rejection of claims 11-17 under 35 U.S.C. § 112, second paragraph, and that a complete response to the rejection has been provided.

35 U.S.C. § 102(e) Rejection Claims 11 and 13-15 – Evans Reference

In numbered paragraph 5 of the Office Action mailed March 29, 2007, claims 11 and 13-15 were rejected under 35 U.S.C. § 102(e) as being anticipated by the Evans reference (US 2003/0087238).

Applicants have amended independent claim 11; therefore claim 11 is now presented in amended form. Claim 14 has been cancelled. Claims 13 and 15 depend from amended independent claim 11. Applicant believes the invention claimed in amended independent claim 11 and dependent claims 13 and 15 is not anticipated by the Evans reference. The standard for a 35 U.S.C. § 102 rejection is stated in RCA Corp. v. Applied Digital Systems, Inc, 221PQ 385, 388 (d. Cir. 1984) "Anticipation is established only when a single prior art reference discloses, either expressly or under principles of inherency, each and every element of a claimed invention."

Applicant points out that the following limitations and step(s) of Applicants' amended independent claim 11 and dependent claims 13 and 15 are not found in the Evans reference:

"a method of producing a DNA molecule of 1-10 kilobases of user-defined sequence from short oligos of length n (n-mers)," or

"virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size where n is an odd number," or

"providing fragments of length n (n-mers) of defined size where n is an odd number that correspond to said virtual fragments," or

"arraying said fragments of length n (n-mers) of defined size where n is an odd number into groups," or

"separating said DNA sequence segments of length n (n-mers) of defined size where n is an odd number temporally," or

"assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in sequence order to produce said DNA molecule of user-defined sequence," or

"wherein said step of separating said DNA sequence segments temporally is accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors," or

"wherein said double-strand DNA molecules of predetermined base-pairs have an overlap length and the length l_c of oligonucleotides starting thermocycle c is computed by the formula: $l_c=n(c-1)+p_1$ for $c>1$, where $p_1=n-v_1$."

Since the step(s) described above are not found in the Evans reference, the Evans reference does not support a 35 U.S.C. § 102(e) rejection of Applicants' amended claims 11, 13, and 15 and the rejection should be withdrawn.

35 U.S.C. § 102(e) Rejection Claims 16 and 17 – Evans Reference

In numbered paragraph 6 of the Office Action mailed March 29, 2007, claims 16 and 17 were rejected under 35 U.S.C. § 102(a) and 35 U.S.C. § 102(e) as being anticipated by the Evans reference (US 2003/0087238).

Applicants have amended independent claim 11; therefore claim 11 is now presented in amended form. Claims 16 and 17 depend from amended independent claim 11. Applicant believes the invention claimed in amended independent claim 11 and dependent claims 16 and 17 is not anticipated by the Evans reference. The standard for a 35 U.S.C. § 102 rejection is stated in RCA Corp. v. Applied Digital Systems, Inc, 221PQ 385, 388 (d. Cir. 1984) "Anticipation is established only when a single prior art reference discloses, either expressly or under principles of inherency, each and every element of a claimed invention."

Applicant points out that the following limitations and step(s) of Applicants' amended independent claim 11 and dependent claims 16 and 17 are not found in the Evans reference:

> "a method of producing a DNA molecule of 1-10 kilobases of user-defined sequence from short oligos of length n (n-mers)," or
>
> "virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size where n is an odd number," or
>
> "providing fragments of length n (n-mers) of defined size where n is an odd number that correspond to said virtual fragments," or
>
> "arraying said fragments of length n (n-mers) of defined size where n is an odd number into groups," or
>
> "separating said DNA sequence segments of length n (n-mers) of defined size where n is an odd number temporally," or

"assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in sequence order to produce said DNA molecule of user-defined sequence," or

"wherein said n-mers are of a size n+1, n+2, etc," or

"wherein said multiplicity of DNA sequence segments comprise oligos in multiple reading frames."

Since the step(s) described above are not found in the Evans reference, the Evans reference does not support a 35 U.S.C. § 102(e) rejection of Applicants' amended claims 16 and 17 and the rejection should be withdrawn.

35 U.S.C. § 103 Rejection – Office Action Paragraph 8

In numbered paragraph 8 of the Office Action mailed March 29, 2007, claims 11, 13-15, and 17 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the Selifonov reference (WO 00/42560) in view of the Evans reference (US 2003/0087238).

Applicants have amended claim 11 and claim 14 has been cancelled. Claims 13, 15, and 17 depend from amended independent claim 11. Applicants believe the invention claimed in amended independent claim 11 and dependent claims 13, 15 and 17 is patentable and that the Selifonov reference and the Evans reference do not support a 35 U.S.C. § 103(a) rejection.

Applicants' Invention of Claims 11, 13, 15, and 17

Applicants' invention of claims 11, 13-15, and 17 is a method of producing a DNA molecule of 1-10 kilobases of user-defined sequence from short oligos of

length n (n-mers). The claimed method comprises a specific combination of steps. The specific combination of steps is not shown or suggested by either the Selifonov reference or the Evans reference. Applicants' original Figure 4 reproduce below and the description of the system 400 from Applicants' original specification illustrate the claimed invention.

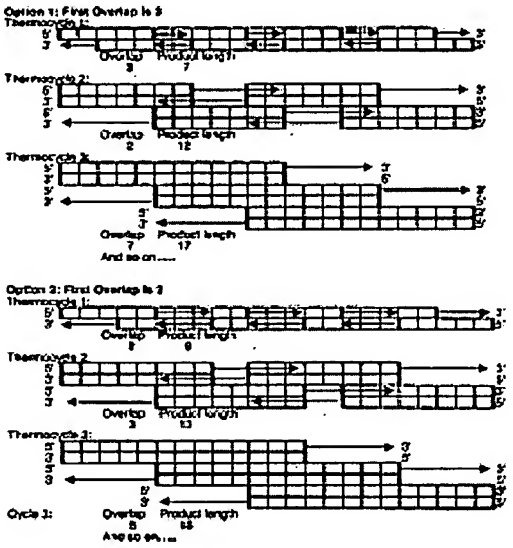


FIG.4

Use of odd-sized starting oligos - Referring now to FIG. 4, another embodiment of a system of creating long DNA sequences, e.g., 1-10 kilobases, from short oligos of length n (n-mers) of the present invention is illustrated. The system is designated generally by the reference numeral 400. The system of parallel synthesis 400 provides a process for making very long (greater than is possible with conventional phosphoramidite chemistry) DNA of user-defined sequence. The method begins by using computational techniques to break the desired sequence into fragments of defined size.

These n-base fragments are then arrayed in groups of n-base oligonucleotides and assembled into double-strand DNA molecules using DNA polymerase. The starting oligos may be of size n, where n is an odd number. The desired, hybridizing overlaps between oligos in the first thermocycle of PCR may be specified by the user. Table 1 gives a few examples of the overlap length, oligo

length, and number of polymerized bases for several scenarios of starting oligo size and overlap in the first thermocycle, and the formula for computing these variables. The products of the reactions in the first tier of PCR reactions (each PCR reaction involves many thermocycles) are then combined, in as many steps as necessary, and assembled by polymerase into still-longer molecules, until the final desired product is assembled. The final product is then amplified using PCR.

The assembly process is substantially the same as the process called DNA shuffling. It is similar to PCR in that there is a template, a primer, a DNA polymerase, and the attendant nucleotides and buffers. It is dissimilar to PCR in that the primer and template are the same entities – the n-mers themselves. Following the parallel assembly process, the final product can be amplified by PCR. Any DNA polymerase commonly used for PCR can be used for this purpose.

The system 400 is similar to the system 300 described above and illustrated in FIG. 3; however, in the system 400, the starting oligos may be of odd length instead of even length. That is, in the system 300, the oligos, or n-mers, are of even length equal to n with a hybridizing overlap between complementary oligos of length n/2 in the first two thermocycles. In contrast, in the system 400, the length n may be odd, and the overlap length between hybridizing oligos may be specified by the user. Given a desired overlap v_1 in the first thermocycle and the length n of the starting oligos that are specified by the user, the length l_c of oligonucleotides starting thermocycle c is computed by the formula: $l_c=n(c-1)+p_1$ for $c>1$, where $p_1=n-v_1$. The length v_c of desired overlap between oligos in thermocycle c is given by $v_c=n(c-2)+p_1$ for $c>1$. The number p_c of bases polymerized in thermocycle c is $p_c= n$ for $c>1$.

Figure 4 illustrates the first three thermocycles for the two scenarios starting with n=5 outlined in Table 1 below. Each yellow box indicates a nucleotide, and a series of yellow boxes represents an oligonucleotide, where the heavy black vertical lines indicate the ends of an oligonucleotide. The 5′ and 3′ ends of the plus and minus strands are labeled, and where nucleotides are in the same column (overlap vertically) and in the right orientation (5′ to 3′ on the top strand, and 3′ to 5′ on the bottom, from left to right), the desired hybridization occurs. Red arrows indicate polymerization (both the direction and the number of polymerized bases) from 3′ ends during the specified thermocycle. In the first case, in which the first overlap v_1=3, polymerization extends each oligonucleotide by p_1=2 bases, and the length of the oligonucleotides starting the second thermocycle is 7 bases. In the second case, the first overlap v_1=2, polymerization extends each oligonucleotide by p_1=3 bases, and the length of the oligonucleotides starting the second thermocycle is 8 bases. These are merely two examples, and any other values of n and v_1 specified by the user may be used.

The Selifonov Reference

The Selifonov reference is International Patent No. WO 00/42560. The system of the Selifonov reference is an "in silico" DNA shuffling technique, in which part, or all, of a DNA shuffling procedure is performed or modeled in a computer system, avoiding (partly or entirely) the need for physical manipulation of nucleic acids. The system is described in the Selifonov reference specification and the Selifonov reference specification Fig. 1A reproduce below.

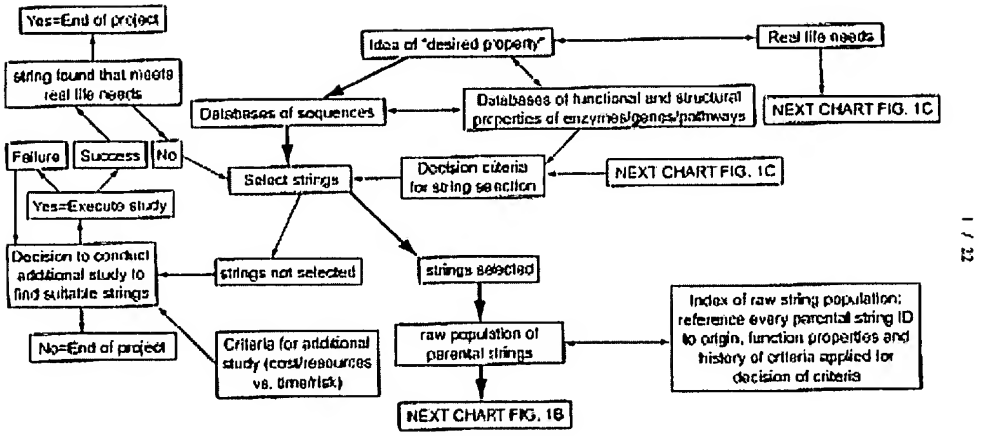


Fig. 1A

A set of flow schematics which provide a general representation of an exemplary process of Directed Evolution (DE) by GAGGS are enclosed (Figs. 1-4). Fig. 1 provides an example decision making process from an idea of a desired property to selection of a genetic algorithm. Figure 2 provides a directed evolution decision tree from selection of the genetic algorithm to a refined library of parental character strings. Figure 3 provides example processing steps from the refined parental library to a raw derivative library of character strings. Figure 4 processes the raw character strings to strings with a desired property.

Generally the charts are schematics of arrangements for components, and of process decision tree structures. It is apparent that many modifications of this particular arrangement for DEGAGGS, e.g., as set forth herein, can be developed and practiced. Certain quality control modules and links, as well as most of the genenic artificial neural network learning components are omitted for clarity, but will be apparent to one of skill. The charts are in a continuous arrangement, each connectable head-to tail. Additional material and implementation of individual GO modules, and many arrangements of GOs in working sequences and trees, as used in GAGGS, are available in various software packages. Suitable references

describing exemplar existing software are found, e.g., at http://www.aic.nrl.navy.mil/galist/ and at http://www.cs.purdue.edu/coast/archive/clife/FAQ/www/Q20-2.htm. It will be apparent that many of the decision steps represented in Figs. 1-4 are performed most easily with the assistance of a computer, using one or more software program to facilitate selection/ decision processes.

The Evans Reference

The Evans reference is United States Published Patent Application No. 2003/0087238 for a method for assembly of a polynucleotide encoding a target polypeptide. The system of the Evans reference utilizes the results of genomic sequence information by computer-directed polynucleotide assembly based upon information available in databases such as the human genome database. The system is described in the Evans reference specification and the Evans reference Figure 5 reproduce below.

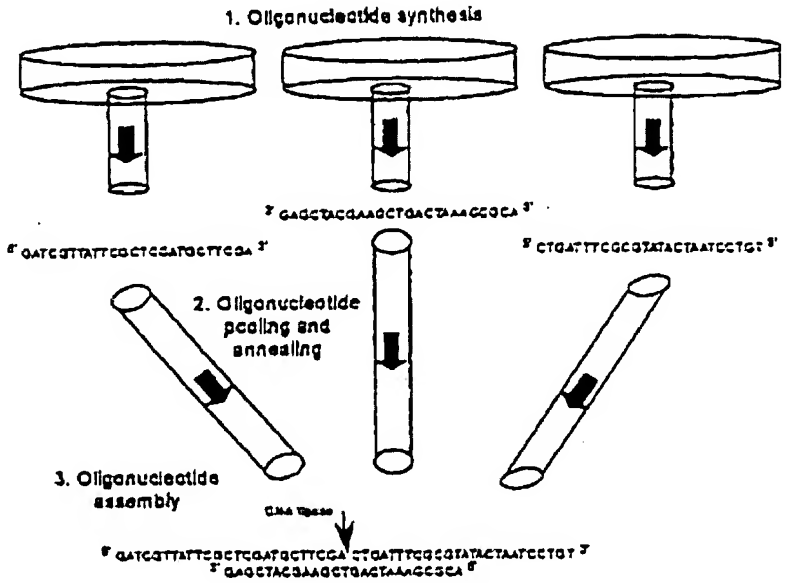


Figure 5

FIG. 5 depicts that oligonucleotide synthesis, oligonucleotide assembly by pooling and annealing, and ligation can be accomplished using microfluidic mixing. As shown in FIG. 5, oligonucleotide synthesis, oligonucleotide assembly by pooling and annealing, and ligation can be done using microfluidic mixing, resulting in the same set of critical triplex intermediates that serves as the substrate for annealing, ligation and oligonucleotide joining. DNA ligase and other components can be placed in the buffer fluid moving through the instrument microchambers. Thus, synthesis and assembly can be carried out in a highly controlled way in the same instrument.

Patentability of Invention Defined by Claims 11, 13, 15, and 17

The Examiner bears the initial burden of factually supporting a *prima facie* conclusion of obviousness. To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Applicants point out that the following combination of steps of Applicants' amended independent claim 11 and dependent claims 13, 15 and 17 are not found or suggested by the Selifonov reference or the Evans reference:

> "a method of producing a DNA molecule of 1-10 kilobases of user-defined sequence from short oligos of length n (n-mers),"

"virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size where n is an odd number,"

"providing fragments of length n (n-mers) of defined size where n is an odd number that correspond to said virtual fragments,"

"arraying said fragments of length n (n-mers) of defined size where n is an odd number into groups,"

"separating said DNA sequence segments of length n (n-mers) of defined size where n is an odd number temporally,"

"assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in sequence order to produce said DNA molecule of user-defined sequence,"

"wherein said step of separating said DNA sequence segments temporally is accomplished by said DNA sequence segments being added gradually, in an order that is predicted computationally to minimize errors,"

"wherein said double-strand DNA molecules of predetermined base-pairs have an overlap length and the length l_c of oligonucleotides starting thermocycle c is computed by the formula: $l_c=n(c-1)+p_1$ for $c>1$, where $p_1=n-v_1$,"

"wherein said multiplicity of DNA sequence segments comprise oligos in multiple reading frames."

Since Applicants' combination of steps of amended independent claim 11 and dependent claims 13, 15 and 17 identified above are not shown by the references it is clear that combining the references would not produce

Applicants' invention. The facts that the two references fail to show the combination of elements of Applicants' amended independent claim 11 and dependent claims 13, 15 and 17 identified above and there is no showing of how a combination would produce Applicants' invention, make it clear that there could not be a combination of the two references that would produce Applicants' invention. Accordingly, the Examiner has not factually supported a *prima facie* case of obviousness and the rejection should be withdrawn.

35 U.S.C. § 103 Rejection – Office Action Paragraph 9

In numbered paragraph 9 of the Office Action mailed March 29, 2007, claim 16 was rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the Selifonov reference (WO 00/42560) and Evans reference (US 2003/0087238) in view of the Murphy et al reference (USPN 6,994,963.

Applicants have amended claim 11 and claim 16 depends from amended independent claim 11. Applicants believe the invention claimed in amended independent claim 11 and dependent claim 16 is patentable and that the Selifonov reference and the Evans reference and the Murphy et al reference do not support a 35 U.S.C. § 103(a) rejection.

Applicants' Invention of Claim 16

Applicants' invention of claims 16 is a method of producing a DNA molecule of 1-10 kilobases of user-defined sequence from short oligos of length n (n-mers). The claimed method comprises a specific combination of steps. The specific combination of steps is not shown or suggested by either the Selifonov reference or the Evans reference or the Murphy et al reference. Applicants' original Figure 4 reproduce below and the description of the system 400 from Applicants' original specification illustrate the claimed invention.

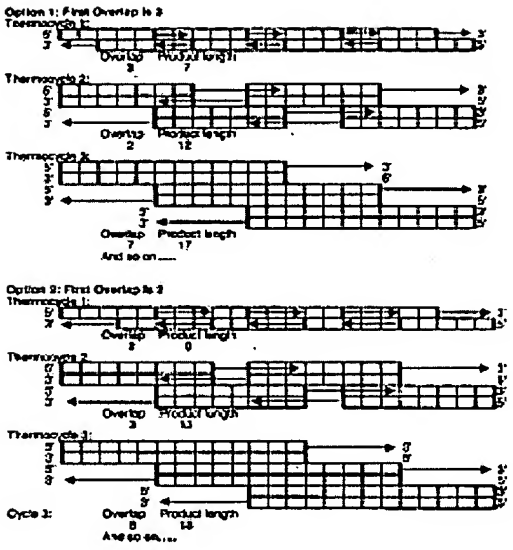


FIG.4

Use of odd-sized starting oligos - Referring now to FIG. 4, another embodiment of a system of creating long DNA sequences, e.g., 1-10 kilobases, from short oligos of length n (n-mers) of the present invention is illustrated. The system is designated generally by the reference numeral 400. The system of parallel synthesis 400 provides a process for making very long (greater than is possible with conventional phosphoramidite chemistry) DNA of user-defined sequence. The method begins by using computational techniques to break the desired sequence into fragments of defined size.

These n-base fragments are then arrayed in groups of n-base oligonucleotides and assembled into double-strand DNA molecules using DNA polymerase. The starting oligos may be of size n, where n is an odd number. The desired, hybridizing overlaps between oligos in the first thermocycle of PCR may be specified by the user. Table 1 gives a few examples of the overlap length, oligo length, and number of polymerized bases for several scenarios of starting oligo size and overlap in the first thermocycle, and the formula for computing these variables. The products of the reactions in the first tier of PCR reactions (each PCR reaction involves many thermocycles) are then combined, in as many steps as necessary, and assembled by polymerase into still-longer molecules, until the

final desired product is assembled. The final product is then amplified using PCR.

The assembly process is substantially the same as the process called DNA shuffling. It is similar to PCR in that there is a template, a primer, a DNA polymerase, and the attendant nucleotides and buffers. It is dissimilar to PCR in that the primer and template are the same entities – the n-mers themselves. Following the parallel assembly process, the final product can be amplified by PCR. Any DNA polymerase commonly used for PCR can be used for this purpose.

The system 400 is similar to the system 300 described above and illustrated in FIG. 3; however, in the system 400, the starting oligos may be of odd length instead of even length. That is, in the system 300, the oligos, or n-mers, are of even length equal to n with a hybridizing overlap between complementary oligos of length n/2 in the first two thermocycles. In contrast, in the system 400, the length n may be odd, and the overlap length between hybridizing oligos may be specified by the user. Given a desired overlap v_1 in the first thermocycle and the length n of the starting oligos that are specified by the user, the length l_c of oligonucleotides starting thermocycle c is computed by the formula: $l_c=n(c-1)+p_1$ for $c>1$, where $p_1=n-v_1$. The length v_c of desired overlap between oligos in thermocycle c is given by $v_c=n(c-2)+p_1$ for $c>1$. The number p_c of bases polymerized in thermocycle c is $p_c= n$ for $c>1$.

Figure 4 illustrates the first three thermocycles for the two scenarios starting with n=5 outlined in Table 1 below. Each yellow box indicates a nucleotide, and a series of yellow boxes represents an oligonucleotide, where the heavy black vertical lines indicate the ends of an oligonucleotide. The 5′ and 3′ ends of the plus and minus strands are labeled, and where nucleotides are in the same column (overlap vertically) and in the right orientation (5′ to 3′ on the top

strand, and 3′ to 5′ on the bottom, from left to right), the desired hybridization occurs. Red arrows indicate polymerization (both the direction and the number of polymerized bases) from 3′ ends during the specified thermocycle. In the first case, in which the first overlap v_1=3, polymerization extends each oligonucleotide by p_1=2 bases, and the length of the oligonucleotides starting the second thermocycle is 7 bases. In the second case, the first overlap v_1=2, polymerization extends each oligonucleotide by p_1=3 bases, and the length of the oligonucleotides starting the second thermocycle is 8 bases. These are merely two examples, and any other values of n and v_1 specified by the user may be used.

The Selifonov Reference

The Selifonov reference is described above in connection with the paragraph 8 rejection.

The Evans Reference

The Evans reference is described above in connection with the paragraph 8 rejection.

Patentability of Invention Defined by Claim 16

The Examiner bears the initial burden of factually supporting a *prima facie* conclusion of obviousness. To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Applicants point out that the following combination of steps of Applicants' amended independent claim 11 and dependent claim 16 are not found or suggested by the Selifonov reference or the Evans reference:

"a method of producing a DNA molecule of 1-10 kilobases of user-defined sequence from short oligos of length n (n-mers),"

"virtually preselecting a multiplicity of DNA sequence segments that will comprise said DNA molecule of user-defined sequence by using computational techniques to virtually break said user-defined sequence into virtual fragments of length n (n-mers) of defined size where n is an odd number,"

"providing fragments of length n (n-mers) of defined size where n is an odd number that correspond to said virtual fragments,"

"arraying said fragments of length n (n-mers) of defined size where n is an odd number into groups,"

"separating said DNA sequence segments of length n (n-mers) of defined size where n is an odd number temporally,"

"assembling said groups into double-strand DNA molecules of predetermined base-pairs using parallel synthesis, DNA shuffling, and DNA polymerase wherein said step of separating said DNA sequence segments temporally and said step of assembling said groups into double-strand DNA molecules of predetermined base-pairs is accomplished by said DNA sequence segments being added gradually, in sequence order to produce said DNA molecule of user-defined sequence,"

"wherein said n-mers are of a size n+1, n+2, etc."

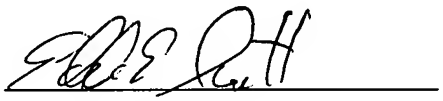
Since Applicants' combination of steps of amended independent claim 11 and dependent claim 16 identified above are not shown by the references it is clear that combining the references would not produce Applicants' invention. The facts that the two references fail to show the combination of elements of Applicants' amended independent claim 11 and dependent claim 16 identified above and there is no showing of how a combination would produce Applicants' invention, make it clear that there could not be a combination of the two references that would produce Applicants' invention. Accordingly, the Examiner

has not factually supported a *prima facie* case of obviousness and the rejection should be withdrawn.

SUMMARY

The undersigned respectfully submits that, in view of the foregoing amendments and the foregoing remarks, the rejections of the claims raised in the Office Action dated March 29, 2007 have been fully addressed and overcome, and the present application is believed to be in condition for allowance. It is respectfully requested that this application be reconsidered, that the claims be allowed, and that this case be passed to issue. If it is believed that a telephone conversation would expedite the prosecution of the present application, or clarify matters with regard to its allowance, the Examiner is invited to call the undersigned attorney at (925) 424-6897.

Respectfully submitted,



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Livermore, California
Dated: June 11, 2007